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Transgenic Animals IQP 2011

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TRANSGENIC ANIMALS

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ABSTRACT

This project attempts to provide updated information on the ongoing research of transgenic animals, and discusses the impact of this technology on society. The project begins by describing the main methods used in making transgenic animals, and then provides examples of these animals and their various classifications. Transgenic technology's many ethical and legal issues are discussed as the project progresses. The authors conclude that this technology provides many medical benefits to society, and it should be continued with strong legislative oversight. Millions of lives can be saved by advances in this field.

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PROJECT OBJECTIVES

The purpose of this IQP is to research the technology of transgenic animals and describe its impact on society. In chapter 1, the main methods of creating and screening transgenic animals are described. Because there are currently so many transgenic animals, their main groups are discussed in chapter 2, and key examples are provided. Chapters 3 and 4 depict the important ethical and legal issues, respectively, of transgenic technology. Special attention is paid to presenting both sides of an ethical argument, prior to making the final author conclusions. In chapter 4, the important Oncomouse court case is discussed, while investigating the pros and cons of animal patenting.

Chapter-1: Transgenic Animal Technology

Randal Bemis

The purpose of this chapter is to explain what transgenic animals are, the methods used to create them, and the screening processes used to identify transgenic positives. For each procedure, alternate methods can be used, and this chapter will aim to present the approaches used by scientists today.

What is a Transgenic Animal?

A transgenic animal is one that carries a foreign gene deliberately inserted into its genome. This technology is used to incorporate DNA that has never existed in these animals before, to give the animal new properties that did not exist in nature. The applications and their benefit to society are widespread. DNA that encodes protein-based medicines or nutrients is being incorporated into cows and goats, which then secrete these proteins in their milk. Other animals are given DNA that will make them grow faster, to make aqua-farming more efficient. Some transgenic animals have DNA that allows them to mimic aspects of human diseases, giving scientists the ability to study the disease process and potentially screen treatments.

Making the Transgene

The newly inserted gene is constructed using recombinant DNA (rDNA) methodology. DNA, the molecule of life, carries information that dictates the properties of living things. Scientists have learned how to isolate DNA, characterize it, excise it, and recombine it, and seal it into other DNAs. Along with the transgene that is intended to be expressed, the inserted DNA often includes other vector sequences (plasmid or virus) that enable it to be incorporated into the

DNA of the host cell. The construct also includes a promoter that controls which tissue the transgene is expressed in (Transgenic Animals, 2011).

When making rDNA, the DNA molecules from two or more sources are recombined to create a single functioning piece of DNA. Using restriction endonucleases, the two desired DNA strands are cut, leave a short overhang that makes the DNA ends compatible with similarly cut DNA. DNA ligases are then used to covalently bond the annealed fragments to each other, forming a single DNA molecule that contains the desired information to be injected into the transgenic animal (Recombinant DNA, 2011). In order for the DNA to be transcribed in the correct tissue, it must have a promoter (**Figure-1**). The DNA also sometimes includes an enhancer sequence that increases the amount of transcription from the transgene. RNA polymerase II, the enzyme that synthesizes RNA from the transgene, binds to the strand of DNA at the start site where the DNA begins to be read and is transcribed into RNA. The basal promoter is located within about 40 base pairs upstream of the start site. It is usually composed of seven base pairs, TATAAAA, and is referred to as the TATA box. Transcription factors (labeled red in figure 1) bind to the TATA box to help control the rate of transcription. Another promoter, the upstream promoter, is located over 200 base pairs upstream of the start site. The upstream promoter helps control tissue specific expression, while the TATA box controls basal transcription. Both promoters are required for correct transcription (Gene Regulation, 2011). The enhancer can be located upstream, downstream, or within the gene that they control. The enhancer binds to transcription factors on the promoter, increasing the speed of transcription (Gene Regulation, 2011).

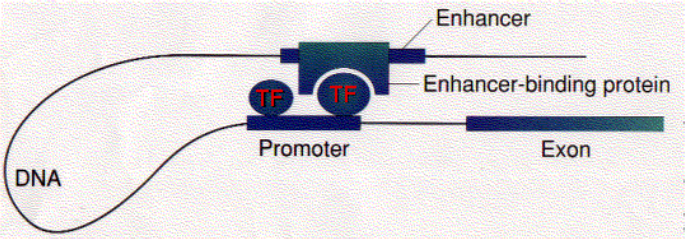

Figure-1: Diagram of Gene Enhancer and Promoter Sequences. These DNA domains act to help facilitate the expression of the transgene. (Gene Regulation, 2011)

Once the desired DNA sequence has been constructed, it is inserted into vector DNA (plasmid or virus) so it can be amplified and inserted into animal cells. The vectors sometimes contain antibiotic-resistance genes to allow cells containing the DNA to be selected in media containing the antibiotic. Once the DNA has been inserted inside an animal cell, by infection when using viruses, or transfection or though electroporation when using plasmids, the vector incorporates into the host genome, and depending on the site of integration hopefully is expressed (Recombinant DNA, 2011).

Creating a Transgenic Animal

Multiple methods exist for creating an animal containing foreign DNA. The two most common techniques are pro-nuclear manipulation and manipulation of embryonic stem cells. Each method has its pros and cons, and is chosen to fulfill specific needs.

Pro-nuclear Manipulation

The most common and reliable technique for creating a transgenic animal is microinjection of DNA into the pro-nucleus of a newly fertilized egg (**Figure-2**). The first step in this procedure is to perform *in vitro* fertilization (IVF) to create a newly fertilized egg. An egg is harvested from a female and fertilized *in vitro*. After the sperm has entered the egg, but

before the male and female pro-nuclei have fused, the male pro-nucleus is injected with the vector DNA containing the transgene. The male pro-nucleus is injected most often because of its larger size and short distance from the surface of the egg. To prevent damaging the newly formed zygote, a micro-pipette with a diameter as small as 2 micrometers is used to penetrate the cell membrane and enter the pro-nucleus. After the injection, the embryo is implanted into the uterine wall of a female of the same species.

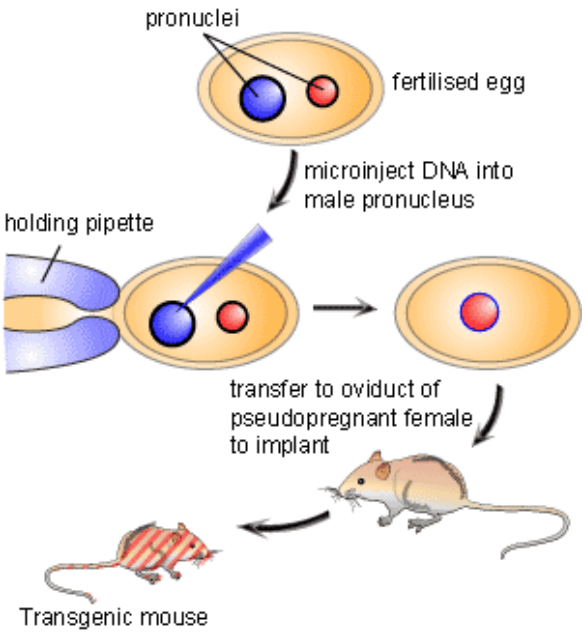

Figure-2: Diagram of Pro-nuclear Manipulation to Create a Transgenic Animal. The male pro-nucleus (blue) in the newly fertilized egg is injected with a DNA vector containing the transgene, and the completed zygote is implanted onto the uterine walls of surrogate female mouse (Walinski, 2004).

The animal that develops is called the founder. Because the DNA was injected prior to the first cell division, all cells of the animal's body should contain a copy of the transgene. If this animal's germ cells contain the transgene, each descendent of the founder will be transgenic (Cartage.org, 2011).

The disadvantage of this technique is the integration of the vector into the animal's genome is random with regards to the location on the chromosome. The location of the transgene

strongly affects transgene expression. So even when a litter of mice were all treated with the same transgenic DNA, The DNA will most likely be expressed differently in each cell. Another problem of random integration occurs when the DNA incorporates into the middle of an already functioning host gene, which can be harmless or fatal. Although it is not the aim of the science, when a transgene integrates and has a noticeable defect, the location of the transgene can help us learn where certain genes are and what they control. The visible defects help scientists map the pre-existing genes and understand the organs or tissues they affect (Cartage.org, 2011).

Even after a successful founder animal has been created, it is not a true homozygote because the DNA has only integrated onto one half of a chromosome. This means that if reproduced with a normal animal, the transgene may not be passed on to some offspring. Because of this, the founder is called *hemizygous*. To produce a homozygous animal for the transgene, two hemizygous founders are mated to produce a homozygous animal with the transgene located on both sides of a chromosome, making all offspring contain the transgene (Cartage.org, 2011).

Embryonic Stem Cell Manipulation

The manipulation of embryonic stem (ES) cells is an alternate method of incorporating foreign DNA into an animal. In comparison to pro-nuclear manipulation, ES cell manipulation targets the animal later in its development, specifically in its blastocyst stage, so not all cells of the founder contain the transgene. To begin the process, an embryo is created by IVF, and grown from 5-6 days to the blastocyst stage from which ES cells are obtained from the inner cell mass. The ES cells are placed in culture, and exposed to the transgene DNA. The treated ES cells are then implanted into the inner cell mass of another blastocyst, which is then

implanted into the uterus of a foster mother, as before. Typically, the foster mother is mated with a vasectomized male to stimulate hormone secretion and prepare the mother for pregnancy, making its uterus more receptive (Transgenic Animals, 2011).

The advantage of ES cell manipulation is it allows targeted integration by homologous recombination. In this process, the transgene construct contains significant regions of host DNA known to flank the desired integration site. During cell division and the cell cycle, the host DNA in the construct recombines with the equivalent host chromosomal DNA, exchanging with it, giving the new DNA its desired location (in an active region).

A second advantage to the ES cell method is it allows for treated ES cells to be selected in culture if they contain the transgene DNA, prior to implantation of the ES cells into the inner cell mass of the blastocyst. To screen for these "positive" cells, the transgene vectors contain genes that alter the way the cells react to certain drugs (Transgenic Animals, 2011). For example, a vector inserted into a mouse ES cell might contain neo^r and thymidine kinase (TK) (**Figure-3**). Neo^r encodes an enzyme that inactivates the antibiotic neomycin (a chemical similar to the drug G418) which kills mammalian cells. TK encodes thymidine kinase which phosphorylates the nucleoside analog ganciclovir, allowing its insertion into DNA, killing the cell (Transgenic Animals, 2011). To screen the ES cells for the desired construct integrated by homologous recombination, the cultured cells are exposed to G418. The cells that failed to incorporate the vector are killed by the G418. Cells that integrated randomly are also killed when exposed to ganciclovir because the TK is expresses. The only cells left in the culture contain the desired genes and neo^r, which incorporated the transgene through homologous recombination (Transgenic Animals, 2011).

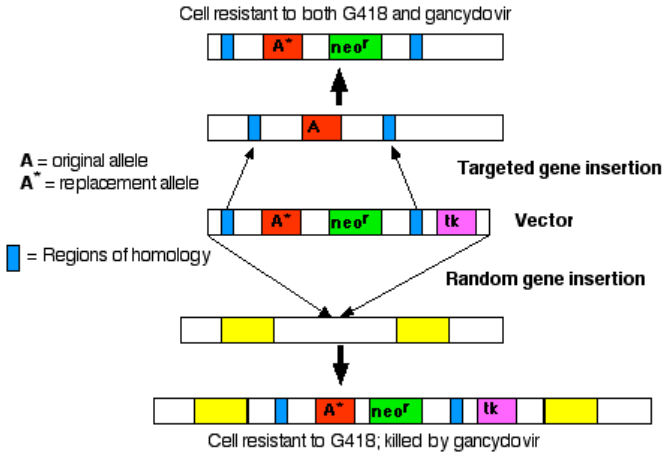

Figure-3: Diagram of Targeted Gene Insertion by Homologous Recombination. In the vector (center), the desired gene (A* red) and neo^r (Green) exist inside the region of homology (blue). On top is the desired outcome where A* is incorporated at the correct location on the chromosome, and botton is an example of random incorporation, placing TK on the chromosome outside the region of homology (Transgenic Animals, 2011).

If the blastocysts develop into living pups, they are tested for the transgene. Only a small percentage of the pups will test positive and of those, all of them will be heterozygous for the transgene. In addition, since the engineered ES cells represent only a minority of the ES cells present in the implanted blastocyst, the animals are chimeric, with some tissues containing the transgene and others not. The heterozygotes are mated, and about one quarter of their offspring will test positive as homozygous for the transgene (Transgenic Animals, 2011).

Knock-out and Knock-in Mice

A common use for targeted integration with ES cell manipulation is creating knock-in and knockout mice. If the desired gene integrated in ES cell manipulation accomplishes homologous recombination, and following subsequent breeding to create homozygous animals,

all cells will have the new transgene instead of the original target gene. In knock-out mice, this targeted gene is now non-functioning. This is useful in discovering what a certain gene does. It can teach us whether some genes are redundant. It can also reveal whether the gene is pleiotropic, meaning expressed in different ways depending on the tissue or stage in development (Transgenic Animals, 2011). For example, if a gene knockout has no effect early in development but does late in development, the gene likely is expressed late in development.

To help facilitate *when* a gene becomes knocked out during development, scientists developed the Cre/*lox*P system. A virus that normally infects bacteria, called P1, codes for an enzyme called Cre recombinase. Cre cuts DNA at LoxP sites. So if the target gene to be knocked out is flanked by LoxP sites (floxed), when Cre is expressed in those cells the gene is excised (knocked out). The Cre gene is attached to a promoter that ensures its expression in the tissue of interest, and only in that tissue does the targeted gene get knocked out. (Transgenic Animals, 2011).

The Cre/*lox*P process can also be used to a gain in function. Some genes are not transcribed in certain tissues because other genes suppress them. So the Cre/*lox*P system can be used to knock out the repressing gene, allowing the target gene to be expressed. Investigators also create knock-in mice by replacing an existing gene with genes they wish to observe under the control of strong promoters (Transgenic Animals, 2011).

Screening Transgenic Animals

The production of transgenic animals is not efficient. In the production of a single transgenic animal line, vast numbers of embryos are created to find one pup that has taken up the transgene in the desired way. Though some transgenic animals can be visibly distinguished, the

majority look like any other member of their species. To make sure the newly bred pups have incorporated a transgene into their genome correctly, they must undergo a screening process. Today's scientists use Southern blot tests or polymerase chain reaction (PCR) analysis to see if pups have incorporated the transgene.

Southern Blot Tests

Using gel electrophoresis and radioactivity, Edwin M. Southern developed the first technique used to sequence DNA, the Southern blot test (Southern, 1975). A Southern blot begins by isolating a sample of DNA from the animal by washing the nuclei in detergents or applying pressure to mechanically force the DNA from the nucleus (DNA Extraction, 2011). Restriction enzymes are used to cut the DNA into fragments of different lengths. These fragments are sorted by length using gel electrophoresis; the fragmented sample is loaded onto an agarose gel and an electrical current is run through it by placing a negative and positive charge on opposite sides of the gel. The DNA has a slight negative charge, attracting it to the positive charged side of the gel. Because the smaller pieces of DNA have less resistance in the gel, they move faster, ending up closer to the positively charged side. Following electrophoresis, the DNA fragment pattern is blotted to a membrane and fixed into place. The membrane is then hybridized to a radioactive single-stranded transgene DNA probe, which hybridizes to the transgene DNA fragment on the membrane if present. The radioactive probe is then identified using x-ray film (Vierstraete, 1999).

Polymerase Chain Reaction (PCR) Analysis

Similar to a Southern blot test, PCR begins with purification of the animals DNA. Then the DNA is placed in a solution containing Taq polymerase and short DNA primers that are complementary to the transgene. The solution is heated to denature the template DNA and then the solution is cooled, allowing the primers to attach to the ends of the transgene if present. The solution is then heated to allow the Taq polymerase to replicate the DNA, starting at the primer sites. This cycle of raising and lowering the temperature is repeated to create millions of copies of the transgene (Brinton and Lieberman, 1999). With a large number of amplified copies, the transgene DNA can be seen in a gel as a thick band (**Figure-4**). The transgene amplified by PCR should move through the gel at the same rate as the known transgene, appearing as lines in the gel with equal distances from the gel origin (The PCR, 2004).

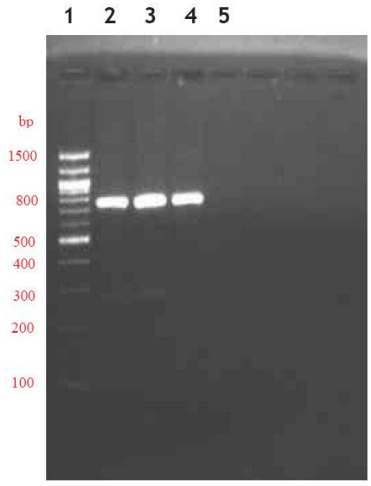

Figure-4: Example Gel Electrophoresis After PCR. The ladder on the left in lane-1 is a size marker. The strong bands in lanes 2-4 represent amplicons, successful PCR reactions. Lane-5 shows a negative PCR reaction (DNA Extraction, 2011).

Sometimes a variation on PCR, termed RT-PCR is used to assay expression of the transgene. This technique is similar to PCR but amplifies a signal from cellular RNA instead of DNA. Once the transgene's correct incorporation into the animal's genome and its expression

has been confirmed, it is ready to be mated to begin a transgenic line that will be used in any number of biological areas where transgenics are used today.

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Chapter-2: Transgenic Applications

Woo Chan Jo

The purpose of this chapter is to investigate the types of transgenic animals created to date. Because transgenic technology is very important medically and biologically, thousands of different animals have been created, so this chapter will group the animals into categories, providing examples within each category.

Disease Models

Disease models are transgenic animals engineered to mimic specific aspects of human diseases to allow their use for developing vaccines or treatments. These models rarely mimic the full symptoms of the human disease, but still allow therapies to be tested against key disease processes.

AIDS Mouse

AIDS (Acquired Immune Deficiency Syndrome) is an immune disorder caused by the HIV virus. As is typical of most retroviruses, the initial infection is often accompanied by a long lag period in which the virus is barely detectable in the blood, so there can be a long lag time before HIV causes severe symptoms. Only in the end stages of infection, as the immune system becomes severely weakened, is the the person considered to fully have AIDS (Avert, 2011).

Most animals do not get AIDS and are not capable of infection with HIV. Some monkeys can be infected with simian immunodeficiency virus (SIV) and have served for years as models for studying how that retrovirus infects the body. But scientists need additional animal

models for studying HIV infection and for screening potential drugs. AIDS mouse is a transgenic animal engineered to express HIV proteins. HIV cannot normally infect mice because they lack CD4 and CCR5 co-receptors on their cell surfaces that HIV binds to cause infection. But mice can be engineered to contain the entire HIV genome and to express it. Malcolm A. Martin, Abner L. Notkins, Jan W. Abramczuk and their colleagues at the National Institutes of Health (NIH) in Bethesda, injected copies of the entire HIV genome into newly fertilized mouse eggs. The eggs were then implanted into surrogate-mother mice. These embryos developed normally and contained the HIV genome in all their dividing cells. Following birth, none of the first-generation transgenic mice showed any symptoms, but surprisingly after they were mated with normal lab mice, some of their offspring showed AIDS-like symptoms, including skin disease resembling psoriasis, pneumonia, and lymphadenopathy. This HIV mouse line has been used for studying the disease, especially the causes of the skin disease and lymphadenopathy (Weiss, 1988).

A different type of AIDS mouse was created without the use of transgenic technology by Dr. J. Victor Garcia. In this model SCID mice lacking an immune system were implanted with human fetal liver and thymus tissue, which contain the proper combination of receptors to be infected by HIV, and which can form portions of a human immune system. The human tissue implants were not rejected because of the faulty immune system. Then the mice were infected with HIV through rectal transmission. The results indicated that six of seven transplant mice showed signs of HIV infection, and three out of four produced antibodies against HIV (from thymus cells). Upon autopsy, HIV was found to be present in the lymph nodes, spleen, other immune tissues, lungs, intestines, and the male and female reproductive tracts (Ambrose, 2007).

Alzheimer's Mice

Alzheimer's disease (AD) is a type of dementia caused by neuro-degeneration. AD is a progressive disorder that worsens over time, affecting memory, thinking, and behavior (PubMed Health 2011a). AD is caused by the improper processing of amyloid precursor protein (APP) on the surface of neurons and glial cells to produce a short peptide fragment amyloid-beta (Aβ) that is highly neurotoxic. The formation of Aβ, or the brain's inability to get rid of it, appears to be the main cause of AD (Access Excellence, n.d.). In some early-onset cases, AD is genetic, with some families having a mutation in the APP gene that increases the rate of Aβ formation.

Animals do not normally get AD. Occasionally orangutans get AD, but it takes decades to see symptoms and they are expensive models. Alzheimer's mice were engineered to initiate AD by inserting the human gene encoding APP in the mouse genome. The version of APP used in the model mimicked an Indiana family with early-onset AD (the Indiana mutation), and was mutagenized by Prof Adams at WPI and his colleagues (Games et al., 1995; King, 1995; Adams, 2010). Within the first six months after the birth of these transgenic mice, there were no symptoms, then from 6 to 9 months the mice started developing Aβ plaques, damaging nerves and synapses (Access Excellence, n.d.). The first successful AD model (Games et al., 1995) resulted from the use of the Indiana APP mutation, used the PDGF-β promoter to drive expression of Aβ in the same areas of the brain as for AD patients, and included introns 6-8 to allow the production of all three isoforms of APP (Adams, 2010). This model was subsequently used by Elan Pharmaceuticals Inc. to create an antibody vaccine to remove Aβ from the brain (Schenk et al., 1999). The vaccinated mice also showed improved behavior (Moran et al., 1995). The AD mouse model has been widely used for attempting to find treatments of what is now the fourth largest fatal disease in America (King, 1995).

Oncomouse

Cancer represents the uncontrolled growth of cells in the body. Normally, cells produced in the body multiply for a finite number of divisions then die, but cancerous cells multiply uncontrollably without dying (PubMed Health, 2011b). Mice engineered to initiate tumors can serve as useful models for studying cancer formation and for screening anticancer drugs. Cancers are sometimes caused by oncogenes, special genes that encode growth factors or signal transduction proteins that cause uncontrolled growth. Placing an oncogene inside the genome of mice can cause the animal to develop cancer.

The first Oncomouse line was created at Harvard University in the early 1980s by Philip Leder and his colleagues (Stewart et al., 1984). This mouse line was engineered to contain the human oncogene *myc*, so the mice develop tumors (**Figure-1**). Harvard and Dupont submitted a patent on the animal in 1984 (Leder and Stewart, 1984), which was finally granted in 1988 as patent number 4,736,866 for the transgenic mouse containing an activated recombinant oncogene in in its germ line and somatic cells. This served as the world's first patent for an animal (discussed in detail in Chapter-3) and noted that the patent did not extend to humans, claiming that such a patent would be unethical. This mouse line is one of the most famous transgenic lines ever created, and serves as a model for studying the formation of tumors and for screening drugs to block cancer formation (Bioethics and Patent Law, 2006). Philip Leder, one of the main creators of Oncomouse described his invention as something that would help the world study cancer and understand it in greater depth (Stern, 2000).

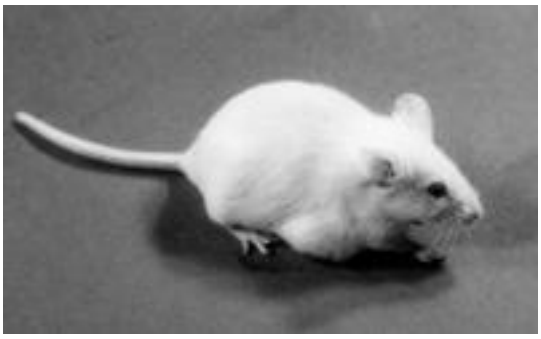

Figure-1: Photograph of Oncomouse.
(Bioethics and Patent Law, 2006)

Transpharmers

Transpharmers are genetically engineered animals that secrete a desired protein drug in their blood, eggs, or milk. The preferred place to produce the protein is in milk, because secretion of a foreign protein in that location is the least likely to have any physiological effects. To accomplish this, the transgene encoding the drug of interest is coupled with a DNA promoter that directs production of the drug only in the mammary gland with secretion into the milk (Biotechnology Information Series, 1995).

Transpharmer Mice

The first transpharmer was a mouse created in 1987 by a group in Framingham, MA. Its purpose was to produce the clot dissolver human tissue plasminogen activator (tPA) in the animal's milk (Gordon et al., 1987). To accomplish this, the cDNA encoding tPA was coupled to a whey acidic protein (WAP) gene promotor. tPA has a crucial role in dissolving blood clots by increasing the breakdown (fibrinolysis) of fibrin-containing blood clots (Abcam, 2011). So this drug is sometimes used to treat heart attack patients to open up clogged heart arteries.

Transpharmer Sheep

After the creation of transpharmer mice, the same idea was used for larger animals such as sheep, goats, and cows. Using nuclear transfer from fetal fibroblast cells, Schieneke et al. (1997) were able to create a transpharmer sheep that produce human clotting factor IX (FIX) in their milk. FIX is essential for blood coagulation, and the use of this protein helps some patients with the disease hemophilia B. The transgene used to make this sheep included the human FIX gene linked with the ovine beta-lactoglobulin gene promoter (so that the production of human FIX takes place only in the mammary gland of the sheep). Using this transgene proved that the production of FIX in both transgenic sheep and mice is possible (Schieneke et al., 1997).

Transpharmer Goats

Scientists at GTC Therapeutics created a transgenic goat that produces human anti-thrombin (ATryn®) in the mammary gland which is then secreted into milk (**Figure-2**). ATryn is a recombinant form of human anti-thrombin, a blood thinning protein, and is the first transpharmed drug to be approved by the FDA. ATryn is used in peri-operative and peri-partum patients to prevent blood clotting. The transgene used to create these goats consists of the human gene encoding anti-thrombin-III fused with a milk protein promoter (ATryn®, 2008).

Figure-2: Photograph of a Transgenic Goat that Produces Blood Thinning Anti-Thrombin Protein it its Milk (BBC News, 2006)

Transpharmer Cows

The most famous example of transpharmer cattle is Herman the Bull (**Figure-3**). Herman was engineered in the Netherlands to produce lactoferrin, a protein with iron that is important for the infant's growth (Biotech Notes, 1994). This bull was created in 1991 by Krimpenfort et al, who used gene microinjection with a transgene that consisted of human lactoferrin cDNA controlled by bovine alpha-S1-casein (Krimpenfort et al., 1991). Cow's milk does not naturally contain lactoferrin, so infants fed mostly cow's milk have to supplement other sources rich in iron. This experiment creates a new source of cow's milk rich in iron. Although Herman himself did not transpharm Lactoferrin, he became the father of eight female calves, and each is able to transpharm lactoferrin (Biotech Notes, 1994).

Figure-3: Picture of Herman the Bull in Naturalis, The Museum of the Netherlands (Naturalis, 2004)

Xenotransplanters

Every day, transplant candidates die waiting to receive organs. Because of the severe shortage of viable organs for transplant, scientists developed the idea of xenotransplantation in which organs would be transplanted from animals into humans until human replacement organs become available. Xenotransplanters are transgenic animals specially engineered to produce organs histocompatible with humans.

Because of genomic similarities, primates at one time were thought to be better candidates for xenotransplanters than pigs, but this was later proven untrue. For example, in the famous case of Baby Fae, on October 26, 1984, in Loma Linda University Medical Center in

California, Baby Fae a five pound infant received a xenotransplant of a baboon's heart into her chest (Fabregas, 2006). Unfortunately, she died after twenty days because her immune system rejected the heart. After this case, the idea of xenotransplantation became very controversial, and the topic was not pursued until the advent of transgenesis.

One of the first transgenic xenotransplanter animals created were pigs lacking the gene (knockout) encoding an enzyme that adds the sugar α-1,3-galactose on the organ surface (Lai et al., 2002). The gene knocked out was α-1,3-galactosyltransferase (GGTA1) (Pearson, 2003). Without GGTA1, the sugar α-1,3-galactose is not added to the organ surface. The sugar is viewed as foreign by humans, so decreasing its concentration on the pig's organ surface decreases the chance of immune-rejection of the transplanted organ. These pigs were engineered in Blacksburg, Virginia, by Revivicor a biotech firm funded by the University of Pittsburg Medical Center (UPMC). Revivicor is planning on having pig organ centers across United States so people on an organ waiting list can have access to the vital organs they need until a human organ becomes available. For further use of these medical wonder pigs, Revivicor is developing two projects that seem promising. One application is to use pig islets to boost insulin production in patients with type-I diabetes. A second application is to use pig hearts for patients with heart failure rather than using mechanical pumps. Revivicor estimates the market for pig organs will eventually be worth about six billion dollars annually (Fabregas, 2006).

However, there are problems associated with xenotransplantation, including the continued need for immune-suppressive drugs, and the danger of virus transmission (Catez, 2005). This topic will be discussed in more detail in chapter-3, but for here suffice it to say that possible viral transmissions from transplanted organs to recipients could be minimized by pre-screening the organs for known viruses prior to transplant and by growing the pigs in relatively

viral free environments, especially indoors. However, dangers remain since previously uncharacterized viruses might not be detected in a pre-screening.

Transgenic Food Sources

The purpose of this category of transgenic animal is to genetically modify animals to better satisfy human consumption needs, by increasing growth rates, reducing carcass fat, and increasing feed efficiency. One way to do this is to give the animal a transgene for a growth hormone. This approach has worked well for fish, but not for mammals. The latter, which caused a lot of problems, resulted in the use of the term "frankenfoods".

Superpig

Super pig is a generic term for at least two types of transgenic animals engineered to produce extra growth hormone. The pigs are also known as the Beltsville pigs because they were created in a research facility in Beltsville, Maryland. The first set of swine was engineered in 1989 to express bovine growth hormone under the control of a mouse metallothionein promoter (Miller et al., 1989). The metallothionein promoter was chosen because it is always on, and does not need to be induced to make its product. The second set was produced in 1997 to express ovine growth hormone under the control of an ovine metallothionein promoter. In both cases, the swine grew to very large sizes. Unfortunately, the production of excess growth hormone produced numerous physiological problems, including kidney and liver problems, uncoordinated gait, bulging eyes, thickening skin, gastric ulcers, degenerative joint disease, heart disease of various kinds, nephritis, and pneumonia (Rollin, 1996; Horwitz, 1998). Thus, the extra growth hormone did not just make big pigs, but also enlarged defective organs and joints (Pursel

et al, 1997). Because of these serious health problems, superpig was euthanized, and scientists have placed a voluntary moratorium banning all growth hormone experiments in mammals.

Superfish

AquaBounty Technologies, headquartered in Waltham, Massachusetts, is a biotech company near to getting FDA approval to sell their genetically engineered (GE) transgenic salmon. If approved, these fish would become the first genetically engineered animals for consumption in the world. These fish, termed AquAdvantage Fish, have been engineered to express salmon growth hormone under the control of an eel-like "ocean pout" promoter. The promoter is switched on throughout all stages of salmon development, so these fish grow faster than wild type salmon whose growth hormone production occurs only during specific stages of development (**Figure-4**). The fish appear to be the same as WT fish in all other aspects of behavior (Biotechnology Industry Organization, 2006). The fish are also sterile, which decreases worries about cross breeding if the fish happen to escape and breed with wild type salmon.

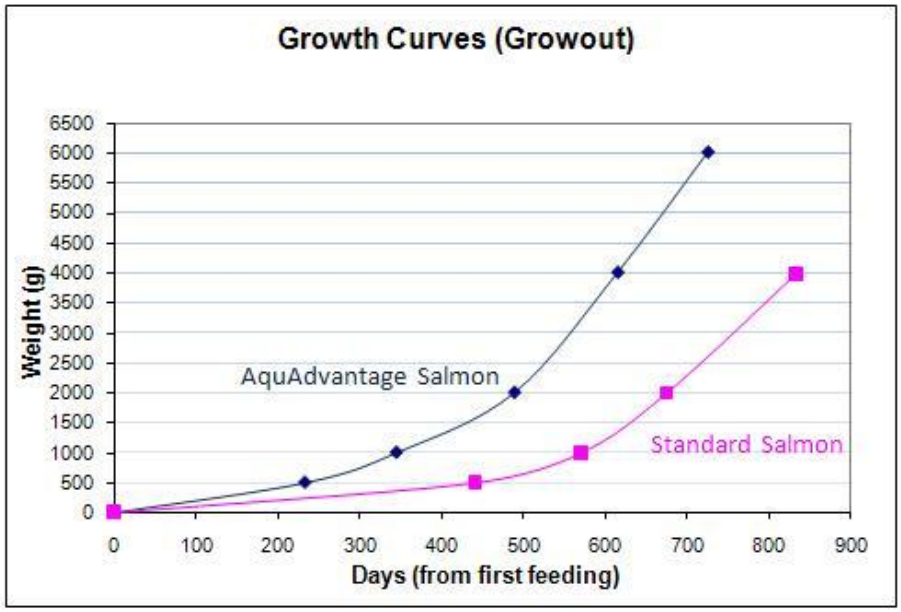

Figure-4: Growth Curves for Transgenic and Wild Type Salmon. (Aquabounty Technologies, 2009)

Biological Models

The last category of transgenic animal is Biological Models. These animals are created to test the function of newly discovered genes and their proteins, or to determine whether a new species can be made transgenic.

ANDi the Monkey

ANDi, which stands for "inserted DNA" spelled backward, is the world's first transgenic monkey (Chan et al., 2001). This monkey (**Figure-5**) was created in Oregon in Gerald Schatten's lab to carry the gene for green fluorescent protein (GFP) to serve as a marker for transgenesis; it did not provide ANDi with any new biological property (Vogel, 2001). Although the experiment was a success since ANDi took up the transgene, the GFP gene was not expressed into GFP protein, which would have fluoresced green. Anthony Chan, one of the researchers creating ANDi said that the ultimate goal of creating such a monkey was to prove that transgenic non-human primates can be made, and if so, they can eventually be used to make disease models closer to humans instead of using mice. To create ANDi, 224 unfertilized rhesus monkey eggs were mixed with a virus containing the GFP gene. Most of the manipulated eggs failed to divide, but 40 of them divided and were chosen at the four-cell stage for implantation into surrogate mothers. These mothers gave birth to three healthy males and two stillborn twins. Of

Figure-5: ANDi the Monkey. Figure shows the world's first transgenic monkey (Vogel, 2001).

the three healthy males, only one (ANDi) carried the GFP gene (Trivedi, 2001).

Smart Mouse

As a scientific model for investigating the function of the protein NR2B, neurobiologist Joe Tsein and his colleagues at MIT created a mouse called Doogie (Tang et al., 1999). This mouse is known for its ability to retain information and solve mazes. The NR2B transgene encodes a subunit of the NMDA brain receptor that predominates when neurons are young. By mimicking young neurons and their ability to make connections, scientists hoped to create a mouse with facilitated learning. Ira Black, Chairman of Neuroscience and Cell Biology at Rutgers University believes that the creation of Doogie will eventually lead to such therapy for humans to enhance our own memory and learning. "But this is far in the future, and is certainly not something we could bring to the bedside tomorrow" (Harmon, 1999).

Figure-6: Doogie the Smartmouse. This mouse shows superior memory and intelligence (BBC News, 1999).

Super Mouse

The world's first expressing transgenic animal was created at the University of

Washington and the University of Pennsylvania by Richard Palmiter and Ralph Brinster who proved that a gene from one animal could be put into another, making the offspring have the transgenic trait (Palmiter et al., 1982). The so-called Supermouse (**Figure-7**) contained a rat growth hormone gene under the control of a metallothionein promoter (always switched on), so the mouse grows to a larger size. Many of their offspring also carried the trait (Klein, 1995). The creation of this mouse proved that transgenic technology was capable of working, and opened the door for the thousands of subsequent transgenic animals created.

Figure-7: Supermouse. Figure shows a photograph of a transgenic mouse (right) containing a growth hormone gene next to one of his non-transgenic littermates (left) (Klein, 1995).

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Chapter-3: Transgenic Ethics

Randal Bemis

The previous chapters discussed transgenic technology, how the animals are engineered and what types have been created to date. The goal of this chapter is to go beyond the technology to explore whether we as a society should be constructing the animals. Transgenic ethics is a balancing act between the benefit to society versus the detriment to the animal. But each category of transgenic animals must be weighed separately, and even specific examples within a category must be considered separately. Not all transgenic applications have negative effects on animals, while other experiments can reduce the animals' quality of life.

Framing the Transgenic Ethics Questions

In life, actions do not always reveal a crisp line between right and wrong, but the positive effects should hopefully outweigh the negatives. With transgenics, the outcome may yield cures for diseases that were once out of reach, but who is to say that a human's life is more important than the animal lives it took to develop these technologies. Scientists, as well as citizens, must look at transgenic technology and weigh the benefits to society against the detriment to the animals to decide what is ethically right and wrong. The positives and negatives of each transgenic application are very different from case to case, so they need to be individually examined to truly judge if a type of transgenic animal should be produced.

Disease Model Ethics

A disease model is a transgenic animal that has been altered to allow them to mimic a human disease, or portions of a human disease. These animals are used to aid our understanding of the disease process, and to help screen potential cures. The benefits to society from these test subjects can be great, but it gives animals a disease their species has never encountered.

Some disease models suffer more than others. In the case of Oncomice, the mice develop tumors which can be painful if allowed to develop to the advanced state. When the experiment requires advanced tumor formation, Animal Care and Use Committees (IACUCs) require the use of painkillers. The American Cancer Society estimates that over ten million Americans suffer from cancer, and the prevalence is similar worldwide. Although no animal's suffering is a good thing, the potential Oncomouse has to help a vast number of people is great.

Another disease model is Alzheimer's disease (AD) mouse. Compared to Oncomouse, AD mice show no evidence of suffering, and the memory loss does not appear to affect the eating behavior, reproduction, or play of the mice. AD mice live mostly normal lives, and have already aided in the the development of an antibody vaccine that removes the senile plaques from the brain that cause Alzheimer's (Schenk et al., 1999).

While many researchers feel that disease models are required for developing therapies, some organizations feel that no animal deserves that treatment. A group called Animal Aid says that animals make poor disease models because altering a small number of genes does not make them human (Animalaid, 2006). While some disease models are not perfect, and some cannot fully simulate a human disease, many have led to advancements in treating diseases like cancer and Alzheimer's (Speaking, 2008).

Transpharmer Ethics

Transpharmers are engineered to secrete protein-based drugs in their milk, blood, or eggs. Transpharmers include mice, sheep, cows and goats, though in most cases drug production occurs in large milk producing livestock. Transpharmers are an example of transgenic animals that live normal lives. Many transpharmers live on farms like any other dairy animal. The mammary glands and milk are not part of the animal's own life supporting organs, so foreign proteins do not have a strong physiological effect on them. As with the production of all transgenic animals, there is a risk of embryo loss when producing a transgenic line. This comes from genes sometimes inserting into non-target areas of the genome, leading to developmental problems. To help minimize this, researchers have developed a targeted method of transgene insertion that uses homologous recombination when treating embryonic stem cells (discussed in Chapter-1). Other researchers are also using adeno-associated viruses (AAV) to increase the accuracy of transgene incorporation. But once a line of transpharmers has been established, the production of protein-based drugs is no different than the production of milk (Gillespie, 2011).

Xenotransplanter Ethics

People die every day waiting for organ transplants to become available, and in many cases a human must die before their organs are available for donation. With xenotransplanters, full organs or cells can be taken from animals and used in humans to help them survive. The animals (usually pigs) are engineered to make their organs more immunologically compatible with human recipients. There are inherent risks involved with xenotransplants, such as rejection and disease transmission, though many people in need of transplants have very few options. The chances of rejection have been reduced using transgenic technology and by using immune

suppressive drugs. And to reduce the chances of disease transmission, donor animals are monitored for known viruses and kept in isolated populations to reduce the risk of disease spread (Carnell, 2000).

Because xenotransplantation incorporates genetically altered tissue into a human, it raises similar ethical concerns as patients receiving pig heart valve transplants. Do these transplants make a person less human or somehow change their identity (Correa, 2001)? Though this could concern a person in need of a transplant, ultimately it should be the patient's choice to accept treatment. If the patient feels that receiving a xenotransplant is wrong, they can deny the procedure.

With respect to the animals themselves, these animals were created to help people. To save human lives, the lives of animals are taken. This is similar to the millions of livestock sacrificed annually for the food industry. Some believe that animal lives hold the same value as humans, while others believe that animal lives must be taken to survive.

Transgenic Food Source Ethics

Superpig and Superfish are two examples of transgenic animals that contain foreign growth hormone genes. These animals were made with the intention of making meat production more efficient. Superpig was considered a failure, while Superfish may soon be the first transgenic food source approved by the FDA.

Superpig is a prominent example of a transgenic animal that is not worth producing because of the strong negative impact the growth hormone had on the animal, while the model provides little benefit to society. Superpig was successful in having faster muscle mass production, but depending on the copy number of the growth hormone gene in the animal's

genome, the side effects were severe and unpredictable. The pigs suffered from kidney and liver problems, as well as lethargy, lameness, uncoordinated gait, bulging eyes, thickening skin, gastric ulcers, severe synovitis, degenerative joint disease, heart disease of various kinds, nephritis, and pneumonia (Rollin, 1996). Because the animal suffering was very clear, researchers voluntarily stopped all projects using growth hormones in mammals, an ethical decision, I, the author, appreciate and respect.

On the other hand, Superfish was a relative success. AquaBounty Technologies developed salmon that grow faster than wild salmon because growth hormones are produced during all stages of development instead of seasonally. This was achieved with minimal detriment to the animal, with side effects including minor gill irritation and a slightly shorter lifespan (Uniqueness, 2002). If the FDA approves the salmon for human consumption, they will change the aqua culture industry forever, being the first genetically engineered meat. This step is important to the field transgenic food sources because the demand for faster food production grows as the world's population climbs.

Biological Model Ethics

Biological models are animals used to discover what certain genes and proteins do in the body. In fact, the world's first expressing transgenic animal was made just for this purpose, to understand the effects of over-expressing growth hormone in mice. Biological models are created so that the over-production or lack of a certain protein can be understood in living animals. With a better understanding, these biological models open up new possibilities for transgenic applications. Though the methods used to create transgenic animals are not efficient, the animals lives used in producing biological models are not wasted. Learning that a certain

gene can hold a negative effect on an animal is valuable. It can prevent future use of certain gene classes in animals, and help prevent further animal suffering by genetic disorders related to that gene.

Activism and Anti-Transgenic Groups

In recent years, transgenic technology has grown, and with it, people's opinions have been formed by different information they hear or read in the media. Public opinion will affect the progression of transgenic technology in the future, thus it is important for the public to understand the technology.

The American Anti-Vivisection Society (AAVS) advocates the reduction of animal use in research, and the use of alternate methods to end animal suffering. Many of their articles are aimed at educating people about the extent of animal testing and what is involved. They aim to steer legislation in a direction that will stop animal suffering. Reducing animal suffering whenever possible is something I admire, but many articles only focus on the animal's health and not on the benefits to humans (AAVS.org, 2011). With the pending approval of genetically engineered fish for human consumption, the AAVS released an article describing the lives of suffering these salmon endure, without mentioning how researchers work to reduce these side effects, and they exaggerated the side effects. The article ends by telling the reader how to contact local officials to prevent the FDA from approving the new technology (AAVS.org, 2011).

While the efforts of groups like the AAVS are aimed to protect animals, the methods they use if bending the truth, are detrimental to scientific research involving animals. Educating the public about these new technologies is important, but needs to involve both sides of the story,

including an unbiased view of true side effects and what benefits the animals bring to society. The AAVS avoids the positive effects gained from the use of disease models, while dwelling on animal suffering without discussing research done to reduce human suffering.

Religious Views on Transgenic Technology

With the rise of transgenic technology, some people look to their religion for how they should feel about it. Though no religion is the same, many share similar opinions on the treatment of animals.

In Christian text, God gave man dominion over animals. This is not to say that man should do whatever they choose with them, but man should understand the value of animals and use them with respect. The Catholic Church has a vast understanding of the use of animals for mankind, and in general is very accepting of it. They make it clear that animals should be used for humans primary needs, and with new technologies, the need for animal use can change. The Vatican says, "Humans must answer to the Creator for the manner in which they treat animals. As a consequence, the sacrifice of animals can be justified only if required to achieve an important benefit for man (Correa, 2001)." This statement is accompanied by the fact that unnecessary animal suffering must be prevented, and consideration for the necessity for certain applications of genetic engineering must be considered.

Buddhist religious beliefs are very different from Christians but reach a similar conclusion on animal research. Buddhists believe in karma. Doing bad things will result in bad karma, potentially bringing bad things on themselves. They also believe that all animals deserve equal treatment. In regards to animal experimentation, the researchers must accept that experimenting on animals may bring them bad karma. Experiments must also be done with good

intentions and only if there is no alternative. Researchers must also do everything in their power to reduce animal suffering and death (Buddhism, 2009).

Other world religions, like Judaism and Islam, come to a similar conclusion about animal research, recognizing animal studies as important if the intentions are good. In all of the world's major religions, doing harm to animals is only right if it is to improve the lives of humans.

Author Chapter-3 Conclusions

The debate on the morality of transgenic research is large and will continue to exist as long as groups promote opposing views on the matter. This debate is fueled by media opinions, and whoever is the most dramatic and outgoing will often stand in the spotlight (Rollin, 1996). Thus, it is important when making ethical judgments about transgenic research to base the opinion on fact and to keep an open mind. Transgenic animals are vastly different in their benefits to society and their extent of suffering, thus it is important to judge each category (and even each specific animal) separately. I agree with opponents that mammalian growth hormone experiments were a disaster, and the self-imposed moratorium was the correct mode of action. Other applications, like AD mouse or transpharmers, appear to have no observable pain, and so far have had strong positive effects on human lives. Some groups believe all harm to animals if wrong, and that no transgenic animal is made without risking the animal's wellbeing. It is important to realize that progress is never made without risks. People take risks every day when driving cars or making investments, but without taking risks, the human race could not have accomplished all the great things they have today. The right thing to do is not always clear, but if researchers do everything in their power to reduce animal suffering, and use transgenic

technology with good intentions, then continuing transgenic animal research is the right thing to do.

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Chapter-4: Transgenic Legalities

Woo Chan Jo

Up to this point we have discussed the techniques used to create transgenic animals, discussed how they are categorized, provided examples within each category, and investigated transgenic animal ethics. This chapter will focus on the legalities of transgenic animals, as an example of how society helps regulate complex technology. We will discuss the landmark transgenic court cases such as the world's first patented animal Oncomouse, both within the U.S. and internationally. The Canadian Oncomouse case is especially important as it concluded that life cannot be patented. We will also discuss the general pros and cons of patenting animals as one of the most debated topics in transgenic animals, and the recent U.S. recommendations on how companies should gain FDA approval for their transgenic products.

General History of Patenting

Patent history goes as far back as medieval times. The very first law gave exclusive rights for a limited amount of time to an inventor and was awarded in Venice in 1473. Later, in England, the government granted sovereign rights to monopolies, allowing them to raise money without forced taxation (A Brief History of the Patent Law of the United States, 2003). For example, a patent was granted for an invention related to the manufacture of silk.

Within the U.S., the *Federal Patent Act* was enacted in 1790 as "An Act to promote the Progress of Useful Arts" (A Brief History of the Patent law of the United States, 2003). The act, containing seven sections, empowered the Secretary of State, Secretary of War, or Attorney General, allowing any two of the three to grant a patent to an invention for up to fourteen years,

as long as the invention was sufficiently useful and important. The 1790 act was later replaced in 1793 by then Secretary of State Thomas Jefferson, who was initially involved with the first act. The new act helped define a "patentable invention" as "any new and useful art, machine, manufacture, or composition of matter, and any new and useful improvement on any art, machine, manufacture or composition of matter", a definition that remains almost unchanged to this day. The 1793 act was frequently amended over the years to address controversial issues, including the patenting of genetically modified animals (A Brief History of the Patent Law of the United States, 2003).

Patentable Inventions, 35 U.S. Code

To grant patents to U.S. inventors, the invention must satisfy three different categories: novelty, usefulness, and non-obviousness. The novelty requirement measures the "newness" of the invention, so the invention must not already be known to the public more than a year prior to the application filing date. The invention must also be able to perform the intended task and satisfy the usefulness requirement. The last requirement is the non-obviousness, so the invention must be "one its kind" and not obvious so any skilled person in the field would have devised the same invention. Furthermore, the 35 U.S. code 101 states "Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title". So the terms "manufacture" and "composition of matter" must be interpreted when attempting to patent life (Bitlaw, 2000).

A patent granted by the U.S. Patent and Trademark Office (PTO) gives rights to the inventor. These rights exclude others from manufacturing or commercializing the patented

product usually for 20 years from the date of application. A patent owner can sell the product or charge others for its use (Bitlaw, 2000).

First Patent on Life: Diamond vs. Chakrabarty

The first patent on a living organism was not an easy trial. The landmark case involved the engineering of a *Pseudomonas* bacterium to allow it to breakdown oil. In 1972, microbiologist Ananda M. Chakrabarty applied for a patent for his invention of a genetically modified bacterium. However, the U.S. examiner initially rejected the patent, claiming that micro-organisms are "products of nature" and are not a "composition of matter".

Chakrabarty appealed the case to the U.S. Supreme Court, making the landmark case of "Diamond vs. Chakkrabarty (1980). The Supreme Court eventually found the invention met all requirements of the 35 U.S.C. 101, so found the microorganism to be a patentable subject matter. The court took into account the definition of manufacture in 35 U.S.C. 101 from the dictionary as: "the production of articles for use from raw materials prepared by giving to these materials new forms, qualities, properties, or combinations, whether by hand labor or by machinery." The court gave expansive meaning to terms such as "manufacture" and "composition of matter" adding an even more comprehensive "*any* manufacture or composition of matter". Chakrabarty's microbe was novel (no one had made an oil eating bacterium before), useful (it could be used to treat oil spills) (**Figure-1**), non-obvious (other genetic engineers had not come up with the idea), and was a new manufacture or composition of matter (which the Supreme Court liberally applied to include engineered living organisms). By allowing the patenting of a microbe, the Supreme Court opened the door to the eventual patenting of engineered animals (Diamond v Chakrabarty, 1980).

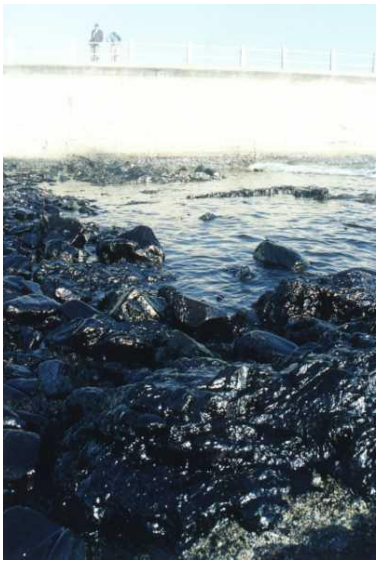

Figure-1: Oil Spill at Mouillie Point. This oil spill accident allowed Chakrabarty's microorganism to meet the patenting usefulness requirement of 35 U.S.C. 101. (Richardson, 2000).

First Animal Patent: Oncomouse

The first animal patent was granted for a mouse termed "Oncomouse" (for its ability to serve as a cancer disease model). The mouse is also known as the "Harvard mouse" because it was invented by Harvard researcher Philip Leder and his collaborators at Dupont Pharmaceuticals (**Figure-2**) (Stewart et al., 1984). The original Oncomouse contained the native mouse c-myc oncogene under control of a mammary tumor viral promoter, which increased expression of the c-myc oncogene in mammary tissue resulting in mammary tumors (Stewart et al., 1984). Later versions of the mouse contained the human *ras* gene which makes it more susceptible to cancer than wild-type mice (Anderson, 1988).

The original patent was filed in 1984 in the United States Patent and Trade Office (Leder and Steward, 1984) for a "transgenic non-human mammal containing a recombinant activated oncogene". That patent was eventually awarded in 1988 as patent #4,736,866. A subsequent

patent was filed in 1992 (Leder and Stewart, 1992) for a method for producing a cell line from a transgenic non-human mammal, which was awarded as patent #5,087,571. A third patent was filed in 1999 (Leder and Stewart, 1999) for a method for testing mice containing oncogenes, which was awarded as patent #5,925,803. These broad patents gave DuPont the right to oppose any entity attempting to use any non-human mammals containing oncogene sequences.

Figure-2: Dupont Logo. This company was awarded ownership of the "Harvard mouse" and is licensed to provide Oncomice for either non-profit or commercial uses (Google.com, 2011).

The patents were very controversial, because for the first time it gave ownership of an animal and its offspring to a company who would control its selling and distribution. DuPont had ownership, and anyone who wished to use the mice for any purposes needed their permission. Initially, DuPont charged very high fees for using the mice, which some researchers argued hindered cancer research rather than stimulate it, because only the wealthy labs could afford to work with the mice. Eventually, DuPont and the National Institutes of Health (NIH) made an agreement to give free access to the mice for non-profit researchers, with companies still paying a small usage fee, and DuPont still required licences for anyone using the mice, and had limits on their breeding and distribution. In 1998, Dupont sublicensed the patent to Tactonic Labs, so Tactonic is able to provide Oncomice worldwide for others to research oncology, study tumor progression, or screen drugs for anti-cancer effects (Tactonic, 1998). Now that Oncomice are more accessible, there is hope to finding new approaches for treating cancer (Smaglik, 2000).

Oncomouse Case in Europe

The Oncomouse patent was not filed just in the United States. The European application was filed on June 22nd 1984 (Sharples and Curley, 2011). The patent was initially rejected by examiners in Munich in 1989 on three grounds: First, patents on plants and animals were forbidden by article 53(b) of the European Patent Convention (EPC). Secondly, the discovery is not reproducible. Third, European patent law does not solve the moral and ethical issues of transgenic animals (Dickman, 1990).

Dupont appealed the rejection, and the Technical Board of Appeal eventually allowed the European patent. The technical board stated that while article 53(b) of EPC excludes the patenting of animal varieties, article 52(1) states that patents are available for inventions capable of industrial application. So the technical board argued that because Oncomice had commercial applications they were patentable. Second, with respect to non-patentable "animal varieties" in 53(b), the board concluded that genetically altered animals like Oncomouse did not constitute "animal varieties", so they were not excluded for patenting by the wording of article 53(b). Finally, the technical board concluded that due to the societal benefits that Oncomice can provide, their invention overrides the ethical issue clause of Article 53(a) which states that European patents will not be granted to inventions contrary to morality. The Board of Appeal can give an exception to specific inventions, and in the Oncomouse case although the mice can suffer if experimenters allow advanced tumor formation, the examiners argued their benefits allowed the exception. They argued Oncomice have "outweighing benefits to mankind", so the European Oncomouse patent was awarded (Sharples and Curley, 2011). One significant difference between the US and European Oncomouse patents is the EPO restricted the patent to

rodents containing oncogenes in 2001, and to mice in 2004, rather than giving ownership to all non-human mammals as in the US case (Cyranoski, 2004).

Oncomouse Patent Denied in Canada

The Canadian Oncomouse patent was filed in June 1985 in the Canadian Intellectual Property Office, but Canada has a strict rule against giving patents for higher forms of life, so did not award the patent. Harvard then requested an appeal to the Commissioner of Patents who also denied the patent on the grounds that a transgenic animal cannot be considered an invention according to the Canada Patents Act (Mitchell and Somerville, 2002). Harvard appealed a second time in 1998, but the denial was upheld by the Canadian Federal Court Trial Division, who stated:

> "On even the broadest of interpretation I cannot find that a mouse is "raw material" which was given new qualities from the inventor. Certainly the presence of the myc gene is new, but the mouse is not new, nor is it "raw material" in the ordinary sense of that phrase… A complex life form does not fit within the current parameters of the Patent Act without stretching the meaning of the words to their breaking point, which I am not prepared to do", said judge Nadon while denying a patent protection for the Oncomouse (Mitchell and Somerville, 2002).

In September 1999, after having made a firm decision to reject the Oncomouse patent, the government of Canada established the "BIOTECanada" biotechnology advisory committee, whose conclusion was that the Canadian denial of the Oncomouse patent or the patenting of any higher life forms would slow scientific advances, especially in creating transgenic animals (Check, 2002).

In August 2000, the Canadian Federal Court of Appeal decided to grant the Oncomouse patent by a vote of two to one, declaring that Oncomouse is a composition of matter. Justice Rothstein concluded that "manufacture" and "composition of matter" should be used more

broadly. Referring to an earlier case where microorganisms were patented, Rothstein used this fact to say the Oncomouse should be patented as well. Rothstein also stated that the term "invention" includes objects that use the laws of nature, and certainly the construction of Oncomouse used "laws of nature" (molecular biology tools) for its creation.

However, in October 2000, the Evangelical fellowship intervened, taking the case to the Supreme Court of Canada. On December 5, 2002, for case file number 28155, the Supreme Court voted 5 to 4 to not award a patent for the Oncomouse, stating that a mouse even with genetic modification is not considered an invention under the Federal Patent Act of 1869 (Mitchell and Somerville, 2002). In the Canadian Federal Patent Act, the word invention is defined as "any new and useful art, process, machine, manufacture or composition of matter, or any new and useful improvement in any art, process, machine, manufacture or composition of matter", and the Supreme Court decided the words "manufacture" and "composition" did not include higher life forms. The word "manufacture" denotes a non-living, mechanistic product or process, so the mouse can not be considered a "manufacture". It also is not a "composition of matter". Justice Michael Bastarache spoke for the majority of the 5-4 vote, saying "Just as 'machine' and 'manufacture' do not imply a living creature, the words 'composition of matter' are best read as not including higher life forms" (Ching, 2003).

Even though the Canadian Supreme Court decided against patenting Oncomouse based on a strict interpretation of their existing Canada Patent Act, the Judges mentioned that this issue must eventually be thought through more broadly (Mitchell and Somerville, 2002).

Animal Patenting Followup

As of the 21st of September 2003, there were approximately 454 animal patents issued in United States (American Anti-Vivisection Society, 2006). Over a fourth of these patents were

funded by federal research, and many required putting a human gene in the animal. 54% of these patented animals serve as disease models, while the rest serve as bioreactors, drug screening models, or for general research (American Anti-Vivisection Society, 2006).

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PROJECT CONCLUSIONS

This IQP gives an overview of the complex technology of transgenic animals and how the technology relates to society. It comprises of four chapters that explain 1) the cloning of the desired transgene DNA, implanting it in a host, and screening transgenic positives; 2) the different applications of transgenic technology, 3) ethical issues and 4) the laws governing transgenic patents. Chapter-1 discusses the two main ways of making transgenic animals, by manipulating the pronuclei of fertilized eggs or by manipulating embryonic stem cells. Both methods require prior cloning of the gene of interest (transgene), which is done through polymerase chain reaction (PCR), and then the transgene is inserted into a vector, such as a virus or plasmid, that helps amplify the DNA. Many embryos are wasted in the process of creating a transgenic animal, making this technology inefficient. To test whether the animal truly has the transgene inserted into their genome, Southern blots or PCR can be used to screen the animals.

There are about five main categories of transgenic animals created to date: disease models, transpharmers, xenotransplanters, food sources, and biological models. Disease models are transgenic animals that mimic a human disease. These models are highly useful for understanding disease processes and for screening potential therapies, but activists say that some models cause animal suffering. This is true only to an extent, as some types of disease models, such as the Alzheimer's mouse, do not suffer by any measurable criterion. On the other hand, the Oncomouse is a good example where the animal can suffer, but the use of regular painkillers,

and euthanizing the animal prior to tumor formation can help minimize animal suffering. Transpharmers are animals bred to produce a desired human protein in their milk, blood, or eggs. These animals serve as useful bioreactors, with no measurable animal suffering. Biological models are animals engineered to over-express or under-express a specific protein to study its effects *in vivo*. Xenotransplanters are engineered to create organs for transplant into humans while on the waitlist for an organ transplant, an idea that animal welfare groups do not agree with. However, the life-saving benefits to society in this case appear to outweigh the sacrifice of the animals. The most unaccepted type of transgenic animal are food sources, as the public is especially in fear of genetically modified foods, in spite the fact that the public has been consuming genetic hybrids (plants and animals) for centuries (although those were created by selective breeding). The first transgenic food source created were the transgenic Superpigs which was a failed experiment, with the animals suffering serious side effects, so we agree with a moratorium on any mammalian growth hormone experiments. However, the transgenic Superfish appear to suffer no such side effects, and Aquabounty's product is near to receiving FDA approval.

Like all very novel sciences that seem to have so much potential, there are many ethical issues with creating these animals. In essence, we must weigh the benefits to society against the detriment to the animal or environment. In general, the authors of his IQP agree that all categories of transgenic animals should continue, with specific exclusions such as mammalian growth hormone experiments, but with strong oversight to minimize animal suffering.

Laws have been created to allow the patenting of transgenic animals. The first life form patented was Chakrabarty's *Pseudomonas* bacterium engineered to digest oil, for use in cleaning up oil spills. The award of this patent paved the way for the first patent issued to an animal, Harvard and Dupont's Oncomouse which is predisposed to cancer. However, not every country allows patenting of animals, Canada currently does not allow patenting of any animals.

Based on the research performed for this project, the authors feel that we should allow most practices of transgenesis, while some should be done with more precaution like the making of transgenic food sources, while others should be outright banned such as mammalian growth hormone experiments. With respect to animal patenting, if it helps a company to advance medical information, it should be allowed, but without the use of extremely high licensing fees which can hinder the smaller labs from using the animals. Overall, we believe that this technology has the potential to save millions of lives and so this type of study should be encouraged worldwide with strong oversight.